Genetic Analysis of Dbp5p, a DExD/H-box Protein Essential for Messenger RNA Export in the Yeast *Saccharomyces cerevisiae*

A Senior Honors Thesis

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by

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Abstract

In eukaryotic cells, messenger RNAs (mRNAs) are synthesized in the nucleus and then exported to the cytoplasm, where translation takes place. The export of mRNAs, which exist in the form of mRNA particles (mRNPs), requires conformational changes and interaction with the nuclear pore complexes (NPCs), the gateway that connects the nucleus and the cytoplasm. An essential DExD/H box ATPase Dbp5p, is thought to mediate the conformational remodeling of mRNPs during mRNA export. However, the precise role of Dbp5p is unclear. Here I show, by conducting synthetic-lethal genetic screens that Thp1p, and most likely also Spt7p, function in conjunction with Dbp5p. Thp1p has previously been shown to be involved in mRNA export. Significantly, both Thp1p and Spt7p are known to participate in transcription elongation, confirming a prevailing view that transcription and mRNA export are coupled *in vivo*. However, so far, there is no report that Spt7p plays any role in mRNA export. My work thus opens a way to investigate how transcription elogation is mechanistically linked to mRNA export, especially in the case of Spt7p.

Acknowledgements

I would like to express my gratitude to all those who gave me the possibility to conduct research. I especially want to thank my project advisor, Dr. Tien-Hsien Chang, whose advice, stimulating suggestions and encouragement helped me in all the time of research and writing of this thesis. I have furthermore to thank Dr. Amanda Simcox and Dr. Venkat Gopalan for being the committee members and providing valuable comments. In addition, I would like to thank my peers for supporting me during hard times and offering suggestions for improvement. Finally, I would like to give my thanks to my parents whose love and encouragement enabled me to complete this work.

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Introduction

One of the defining features of eukaryotic cells is the separation of nucleus and cytoplasm by a nuclear membrane. Because messenger RNAs (mRNAs), which carry the genetic information transcribed from DNA, are first synthesized in the nucleus and then translated into proteins in the cytoplasm, they need to be transferred from the nucleus to the cytoplasm. This export process is a remarkably complicated and active process. For example, it has been estimated that more than one million macromolecules per minute are actively transported between the nucleus and cytoplasm of a growing mammalian cell (Ohno *et al.*, 1998). Compounding this problem is the fact that mRNAs are always coupled with numerous proteins to form the mRNA-protein particles (mRNPs) and these large macromolecule complexes (approximately 270nm in diameter) must be squeezed through a narrower passage (approximately 100nm in diameter) termed nuclear pore complex (NPC) to reach the cytoplasm. Exactly how eukaryotic cells accomplish this feat is largely unknown. However, it seems reasonable to assume that mRNPs must undergo conformational changes and specific enzymes are required for performing this task. My research project in the Chang Laboratory deals with an enzyme, Dbp5p, which may play such a role in this process.

Dbp5p, a member of the DExD/H-box protein family, was shown to be required for mRNP export (Tseng *et al.*, 1998) on the basis that its loss-of-function mutations result in rapid accumulation of mRNAs in the nucleus. In addition, the cellular distribution of Dbp5p offers another hint of its role in mRNA export. Dbp5p was found to localize predominantly at the cytoplasmic side of NPCs and in the cytoplasm (Tseng *et al.*, 1998). Thus, it was speculated that Dbp5p plays a role at a later stage of the export process. Since DExD/H-box proteins, which share a DEAD (Asp-Glu-Ala-Asp) signature, are capable of unwinding RNA duplexes and altering RNP structures *in vitro*, it was proposed that Dbp5p participates in the "remodeling" of the RNPs by

utilizing energy derived from ATP hydrolysis to promote mRNA export. Indeed, recent work further supported the idea that Dbp5p is required for mRNP rearrangement (Lund and Guthrie, 2005). Interaction with other proteins, such as Gle1p, a component of cytoplasmic filaments, and InsP, small molecule of phosphoinositide, are important for Dbp5p activity (Abel et al. and Christine et al., 2006). However, the precise functions and mechanistic role of Dbp5p remain to be defined.

The functions of Dbp5p appear highly conserved; there are potential homologues found in various eukaryotic cells such as *Saccharomyces cerevisiae* (the budding or baker's yeast), and human. I used budding yeast as a model system to study Dbp5p's function. The advantages of using yeast as a model system are many. For example, yeast can be easily cloned, its genome is completely sequenced and, most importantly, it shares numerous biological properties that are also present in the higher eukaryotic systems.

I have been pursuing a deeper understanding of Dbp5p's function by employing a powerful "synthetic-lethal" genetic approach. The underlying rationale is to identify specific mutations (the "2^{nd}" mutation) which, when placed in conjunction with a partial loss-of-function mutation (in this case, the *dbp5-1* mutation; or the "1^{st}" mutation) will result in cell death. It has been shown in numerous cases that these two mutations (i.e. 1^{st} and 2^{nd}) almost always define two components that work in concert in the same biological pathway and, in some cases, they even physically interact with each other.

Specifically, this genetic scheme works as follows. I started out with a yeast strain, YTC1104, in which a wild-type *DBP5* gene and a *dbp5-1* mutant allele are maintained separately on two different plasmids and *dbp5* was deleted on the chromosome (Fig. 1, top circle). Because *dbp5-1* is a partial loss-of-function allele, the *DBP5*-carrying plasmid can be freely lost without consequence.

To identify the second mutation [or the synthetic lethal (i.e. SL) mutation], I used UV-light to generate random mutations in the starting strain. These mutations, if present in specific components that work together with Dbp5p, will make it imperative for cells to retain the *DBP5*-carrying plasmid for viability. Importantly, this type of mutant cannot grow on plate containing 5-fluoroorotic acid (5-FOA) (Fig. 1; right circle, second row).

I have successfully isolated several recessive mutations that have the properties exactly as I anticipated. Work has been done to clone the wild-type genes corresponding to several synthetic-lethal mutations on the basis that acquisition of a wild-type copy of the corresponding gene in the mutant cells will relieve the synthetic-lethal phenotype. At least one of the genes identified, *THP1*, is known to play a role in mRNA export. Interestingly, *THP1* and the other gene, *SPT7*, which is in the process of being validated, are both involved in transcription elongation.

Materials and Methods

Yeast Strains and Plasmids

The yeast strains and plasmids used in this study are listed in Table 1.

Media

YPD, SD, and LB media and plates were prepared using standard recipes. 5-FOA plates were made in SD at a concentration of 1 mg/ml. Ampicillin was used at a final concentration of 0.2 µg/ml. G418 plates was made by adding 100 µl of 50 mg/ml G418 stock onto a YPD plate.

Mutagenesis and Isolation of Mutants that require *DBP5* for growth

Strain YTC1104 was mutagenized by 30 seconds irradiation of ultraviolet light to reach ~10% survival rate. Mutations that were synthetic lethal to *dbp5-1* was screened using the adenine red-white sectoring assay (Bender and Pringle, 1991) and 5-FOA counter selection

method. The criteria of *sl* mutant candidates were the red color phenotype on YPD and non-viable phenotype on 5-FOA.

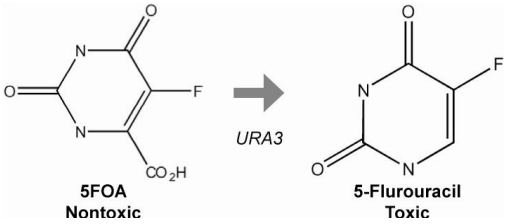
Genetic backcrosses for sl27 and sl64 crossing to YCT280 have been done three times by dissecting tetrads to clean up the background. Tetrads that required DBP5 for growth were used in later analysis.

► Adenine red-white sectoring assay:

…… ⟶ intermediate X ⟶(Ade3p) intermediate Y ⟶(Ade2p) …… ⟶ Adenine

The color phenotype of red or white yeast cell is determined by the presence of the accumulation of red pigment (shown above as intermediate Y). The ade2 yeast cells accumulate a red pigment whereas ade2 ade3 cells do not, because the ade3 mutation blocks the pathway at previous point and only the colorless intermediate X is accumulated. A sectoring colony containing both red and white color indicates the ADE3 plasmid is not essential in the certain strain. However, an ade2 ade3 strain carries ADE3 plasmid forms completely red colonies if the plasmid is required for cell viability. Cells from red colonies without white sectors were streaked out on YPD. Only stains that give red color on every colony were determined to be ADE3 plasmid dependent.

► 5-Fluoroorotic Acid (5-FOA):



5-Fluoroorotic acid (5FOA) inhibits cell growth when organisms utilize orotic acid as a source to form pyrimidine rings. In *Saccharomyces cerevisae*, the uracil biosynthesis pathway contains orotic acid as an intermediate; therefore, a *ura3* mutant strain can grow on 5FOA

whereas growth of wild-type URA3 is inhibited (Boeke *et al.*, 1987). In this study, selection of cells that were dead on 5FOA was employed to screen mutants that require *DBP5* for growth.

Electroporation of *E.coli*

Electro-competent cells were exposed to 1.25 kV pulse for 4.5 msec. Addition of SOC and incubation at 37 ^{o}C followed.

Restriction Enzyme Digestion

The digestion reactions were set up by mixing DNA, 10X buffer, 10X BSA as applicable, enzyme and water. The amount used of each reagent varied in different case to fulfill the purpose. Reaction time varied from an hour to overnight incubation at 30 ^{o}C.

Cloning

Primers were designed as 30-mers that consisted of 16-18bp the sequence near a target gene (at approx. 700 bp upstream or 300 bp downstream of a target gene), 6-8bp of enzyme recognition sequence, and 6bp random sequence at the end. The PCR products of the corresponding wild-type genes were ligated into pRS315 through NotI and SacII sites.

Diploid construction and tetrad dissection

Cells of each haploid parent were mixed on YPD plate for more than 4 hours. Cell mixture was diluted to form single colonies and replica-plate onto both YPD plate with either a lawn or α lawn to identify diploids. Diploid cells were first grew again on YPD overnight and shift to a poor environment (1% potassium acetate) for 2-3 days. Tetrads were then treated with glusulase and dissected under the microscope.

Results

Synthetic-lethal screen

Before I started the synthetic-lethal screen, a starting strain for this purpose had been constructed. This haploid strain has the following features. First, the chromosomal *DBP5* gene has been deleted and replaced by an deletion allele marked by the *HIS3* selectable marker (*dbp5*Δ*::HIS3*). Second, it contains an *URA3/ADE3*-marked plasmid on which a wild-type *DBP5* gene is carried. Third, it carries a second plasmid marked by *TRP1* that harbors a temperature-sensitive allele of DBP5, i.e. *dbp5-1*, which has been previously isolated in the Chang Laboratory. So, if this strain is grown under non-selective conditions, both or either one of the plasmids can be lost. Losing both plasmids will result in cell death, because there will be no *DBP5*, which is an essential gene. Loss of the plasmid containing *dbp5-1* would leave the cell with the *DBP5* plasmid, so the cell should behave like the wild-type cell. Losing only the plasmid containing *DBP5* would still be tolerable, because *dbp5-1* on the other plasmid is still functional at the permissive temperature, e.g. 30˚C, to keep the cell alive. The synthetic-lethal screen seeks to isolate mutations that require a copy of wild-type *DBP5* to be viable.

After UV mutagenesis, candidate mutants were identified on the basis of the two following criteria: capable of forming exclusive red colonies and the inability to on 5-fluoroorotic acid (5-FOA) plate. The underlying rationale was that if the cell cannot lose the *DBP5/ URA3/ADE3* plasmid, then the colony would be completely red on YPD plate because the red pigment intermediate accumulates when the cell's genotype is *ADE3* and *ade2*. In addition, cells that carried *URA3* would be dead on 5-FOA-containing plate due to the formation of toxic compound, fluorouracil, during the uracil synthesis, produced by the Ura3p. By screening through three batches of UV-mutagenized cells, a total of ~387,000 colonies, I have successfully isolated 5

mutants that could not grow on plate containing 5-FOA and were red/non-sectoring on YPD plate. These 5 mutants were named as *sl22*, *sl27*, *sl43*, *sl47*, and *sl64*.

Synthetic-lethal specificity to *dbp5-1*

After obtaining these mutants, I set out to examine whether the observed synthetic lethality was due specifically to the interaction between *dbp5-1* and the uncharacterized mutations. Mutant strains were transformed with plasmids containing either wild-type *DBP5* or mutant *dbp5-1* genes. A mutant is judged to harbor a mutation specifically synthetic-lethal to *dbp5-1*, if it can regain the red/white sectoring phenotype, i.e. the ability to lose the original *DBP5/ADE3/URA3* plasmid, and grow on the 5-FOA plate, upon receiving another copy of *DBP5* plasmid. In addition, such regaining of the sectoring phenotype and the ability to grow on the 5-FOA plate should not occur upon receiving another copy of the mutant *dpb5-1* gene. At the end, these 5 mutants were determined to be carrying mutations specifically synthetic lethal to *dbp5-1*. Fig. 2 shows an example of the *sl4*3 mutant, which displays a mixture of red and white colonies when received an extra copy of *DBP5*. Qualified candidates were then subjected to further genetic analysis.

Dominance and recessiveness

Because my goal was to eventually clone the genes corresponding to the *sl* mutations, I also determined the dominant/recessive property of the *sl* mutations. Recessive mutations will be easier to deal with in terms of cloning their corresponding genes, because yeast genomic libraries made from the wild-type strain, which carry the dominant wild-type allele, are readily available for cloning experiments. To know whether a particular mutation is dominant or recessive with respect to its wild-type allele, I crossed the mutant strains with a wild-type tester strain of the opposite mating type and analyzed the phenotype of the resulting diploid strain (see Materials and Methods). If the diploid strain exhibits the same phenotype as that of the mutant strain (i.e. inviable on

5-FOA), then the mutation is dominant. On the other hand, if the diploid strain is viable on 5-FOA, then the mutation is recessive, because the wild-type allele is dominant over the mutant allele, allowing a wild-type growth phenotype. Our analysis showed that 3 out of 5 mutants were recessive (i.e. *sl27*, *sl43*, and *sl64*), while the remaining 2 were dominant (i.e. *sl22* and *sl47*).

Cloning the genes corresponding to the synthetic-lethal mutations

To clone the genes corresponding to the synthetic-lethal mutations, I transformed the mutant strains with a yeast YCp50 library, which is a genomic library built on the plasmid backbone that has a yeast centromere and a *LEU2* selectable marker (Fig.3). These library plasmids typically contain yeast DNA inserts about 9—12 Kb in length. Transformants that grew on the 5-FOA plate upon gaining YCp50 plasmids were isolated and their plasmid DNAs were extracted for further analysis. Two types of plasmids could have been recovered: the transformed library plasmid (i.e. YCp50/*LEU2* plasmid) and the *dbp5-1/TRP1* plasmid originally existing in the mutant strains. To focus on getting back the library plasmids, I transformed the recovered DNA (from yeast cells) into an *E. coli* strain, MH1066, which harbors a *leuB600* mutation that can be complemented by the yeast *LEU2* gene, which is carried on the library plasmid. *E. coli* transformants were initially selected on the LB-Ampicilin plate and then replica onto M9 medium without leucine. Plasmids were then recovered from colonies grown on the M9-Leucine plate.

Synthetic lethality could be relieved by either the wild-type *SL* gene or *DBP5*. Both genes are present in the genomic library. To distinguish these two possibilities, the restriction patterns of the plasmids were compared to that of a control *DBP5*-containing plasmid. Those yielded signature bands found in the *DBP5* digestion were judged to containing *DBP5* and therefore were no longer pursued. For example, digestion by AvrII and HindIII on *DBP5*-containing plasmid yielded a band of 1.09kb (second lane, Fig. 4a.), and by PstI and HindIII yielded a band of 1.34kb (fifth lane,

Fig. 4a.). These bands were also found in some recovered plasmids, e.g. lane 1, in Fig. 4a. Double digestions suggested that the obtained *sl43-1* YCp50 was actually a wild-type *DBP5* clone. Other sl64-YCp50 and sl43-YCp50 restriction patterns by PstI+HindIII were shown in Figure 4. By studying the restriction map, we had knowledge that empty YCp50 plasmids gave three bands at 0.77, 1.8, and 5.81 Kb. Empty plasmids would then be eliminated from the identification process. Only clones that were confirmed not a *DBP5* and had YCp50 patterns, would proceed to re-transformation verification. There were two sl27 YCp50 (number 1 and 4; not shown in the figure), eight sl43 YCp50 (number 7, 8, 12, 15, 13, 18, 26, and 27), and seven sl64 YCp50 (number 4, 5, 6, 8, 11, 12, 14) clones went on to be transforming back to the corresponding sl strains. As a result, *sl43-15* and *sl64-4* YCp50 could always rescue re-transformed cells (i.e. cells were viable on 5-FOA). Because the rescuing ability came from the transforming DNA which included genes other than *DBP5*, we could then sequence the DNA and identify possible wild-type genes corresponding to *sl* genes.

By DNA sequencing using YCp50 primers at two ends of the insert, the genomic regions of the insert were successfully revealed. Results showed that a 11kb DNA on chromosome II (399735-410233), a 12kb DNA on chromosome XV (184857-196810), and a 11kb DNA on chromosome III (189784-201218) may contain the corresponding wild-type genes for *sl27*, *sl43*, and *sl64*, respectively. The most possible corresponding wild-type genes to the mutations were shown in Fig. 5. We chose these genes based on researching on their functions and suggestions from Dr. Chang. At this stage, we thought the synthetic lethal genes for sl27 was *spt7*, for sl43 was *thp1*, and for sl64 was *fen1*, *rrp43*, or *bud5*. To test the hypothesis, individual genes need to be cloned into plasmids and exam the ability to rescue after re-transformation.

I have found that *THP1*, which is known to participate in transcription and mRNA export, corresponds to the *sl43* mutation given the fact that the re-transformation of cloned wild-type gene (*THP1* only) can rescue the sl43 mutant strain. Likewise, *SPT7*, a gene involved in transcription elongation, was suspected to correspond to the *sl27* mutation. However, sl27 transforments failed to grow healthily for many attempts so that the exact identity of *sl27* was not yet so sure. The results of sl64 strain re-transformation showed that the genomic background was too high to identify the *sl64* mutation since the observation of negative control (59% growth on 5-FOA) was not reasonably clean. These results were shown in Figure 6.

The finding thus predicted that strain harboring both *dbp5-1* and *thp1*Δ (Δ: deletion) alleles should not be viable. To test this hypothesis, YTC279 (*dbp5*Δ*::HIS3*) and YTC1204 (*thp1*Δ*::KAN*) were crossed and tetrad dissection had been done to select a spore containing both *dbp5*Δ and *thp1*Δ and at the same time keeping its copy of *DBP5* on a *URA3* plasmid. The candidate spore was HIS^+ and $G418^+$, which indicated *dbp5*Δ and *thp1*Δ on chromosome. For the candidate spore, the *DBP5/ URA3* should not be lost because cells need at least one copy of DBP5 allele to live. Therefore, URA^+, and $5FOA^-$ candidate was recognized. In this strain, the acquisition of a pCA5022 can rescue the cell on 5FOA, whereas gaining of pCA5024 cannot (Figure 7). This result clarified the synthetic lethality of *thp1*Δ to *dbp5-1*.

Discussion

Genetic information carriers, messenger RNAs (mRNAs), need to be exported from nucleus to cytoplasm for all eukaryotic organisms. This export process is a complicated process requiring the participating of numerous proteins. The formation of export mRNPs requires proper recruitment of proteins to facilitate the process. The metabolism of mRNAs entails a multiple-step mechanism which includes transcription, splicing, export, translation, and degradation; some of these steps are coupled. For example, a protein involved in an early stage of spliceosome assembly has direct interactions with the export factor that recruits export proteins to form mRNPs thus indicating a functional coupling between mRNA splicing and export. Also, functional links between transcription and mRNA export have been demonstrated. (Rodriguez, *et al.*, 2004)

The connections between transcription and mRNA export were further drawn by Rodriguez-Navarro (2004) stating that Sus1p-Thp1p-Sac3p complex is functioning as an adaptor between the SAGA complex, a histone acetylase complex in transcription, and NPC-associated mRNA export machinery. Interestingly, the *dbp5-1* synthetic lethal genes, *THP1*, involved in both transcription and mRNA export, and *SPT7*, which encodes a subunit of SAGA protein complex, are thought to take part in mRNA export as *DBP5* does. In this study, we provide supporting evidence that appears to be consistent for the functional coupling of transcription and mRNA export.

To investigate the *SL* gene individually, the suspected *sl27*, corresponding to *SPT7*, is involved in the complex that regulates yeast transcription. We thought it would be interesting if the synthetic lethality was truly due to a *spt7* mutation. The construction of a double deletion strain (i.e. *dbp5*Δ and *spt7*Δ) is ongoing and we hope the resulting strain can confirm the synthetic lethality of *spt7*Δ to *dbp5-1*.

THP1 was identified to correspond to the *sl43* mutation. Also, the synthetic lethality of *thp1* to *dbp5-1* was showed in this study given the fact that the acquisition of *THP1* can rescue the sl43 mutant strain. Because loss-of-function mutation, *dbp5-1*, resulted in rapid accumulation of mRNAs in the nucleus, we anticipate that *thp1* mutation alone should have a similar phenotype as that of the *dbp5-1* mutation. The standard *in situ* hybridization can detect the accumulation of mRNA (or total poly[A]$^+$ RNA) in the *thp1* mutant.

In terms of Dbp5p localization, as stated in the introduction, we were speculating that Dbp5p plays a role at a later stage of the export process. Recent report showing that Dbp5p functions in translation termination (Gross, *et al.*, 2007) may explain the predominance of cytoplasm-located Dbp5p particles. However, Dbp5p also plays role inside the nucleus. For instance, Dbp5p at the nuclear side of NPC was shown as an inactive enzyme that later mediates the export process (Alcázar-Román, *et al.*, 2006). In addition, the genetic and physical interaction of Dbp5p and transcription factor TFIIH revealed the association of Dbp5p with transcription (Estruch and Cole, 2003). Due to these relationships, my next hypothesis is that Dbp5p inside the nucleus interacts with Thp1p to facilitate the mRNA export.

To test the hypothesis, we first need to find the possible physical interactions between Thp1p with Dbp5p. One way to do this is to use the yeast two-hybrid system. It is a molecular genetic tool if two proteins interact, then a reporter gene is transcriptionally activated. The activation of *lacZ* gene can be determined by color reaction on specific media. The other way to test the interaction relationship *in vitro* is the "pull-down" experiment, a form of affinity purification, in which Dbp5p and THP1p will be purified and tested for their interaction. The same experimental scheme could be done for Spt7p to further understand the functional role of Dbp5p.

References

Alcázar-Román, A. R., *et al*. (2006). Inositol hexakisphosphate and Gle1 activate the DEAD-box protein Dbp5 for nuclear mRNA export. *Nat. Cell Biol.* **8**, 711 – 716.

Bender, A. and Pringle, J. R. (1991). Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in Saccharomyces cerevisiae. Mol. Cell. Biol. **11**, 1295-1305.

Boeke, J.D., Trueheart, J., Natsoulis, G., and Fink, G.R. (1987). 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* **154**, 164-75

Brew, C. T., Huffaker, T. C. (2002). The yeast ubiquitin protease, Ubp3p, promotes protein stability. *Genetics* **162**, 1079-1089

Estruch, F., and Cole, C. N. (2003). An early function during transcription for the yeast mRNA export factor Dbp5p/Rat8p suggested by its genetic and physical interactions with transcription factor IIH components. *Mol. Biol. Cell* **14**, 1664–1676

Fischer T, *et al.* (2002). The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. *EMBO J* **21**, 5843-52

Gross T, *et al.* (2007). The DEAD-box RNA helicase Dbp5 functions in translation termination. *Science* **315**, 646-9

Gallardo, M., and Aguilera, A. (2001). A new hyperrecombination mutation identifies a novel yeast gene, THP1, connecting transcription elongation with mitotic recombination. *Genetics* **157**, 79-89

Lund, M. K., and Guthrie, C. (2005). The DEAD-box protein Dbp5p is required to dissociate Mex67p from exported mRNPs at the nuclear rim. *Mol. Cell* **20**, 645-51.

Ohno, M., Fornerod, M., and Mattaj, I. W. (1998) Nucleocytoplasmic transport: the last 200 nanometers. *Cell* **92**, 327-336.

Rodriguez, M. S., Dargemont, C., and Stutz, F. (2004) Nuclear export of RNA. *Biol. Cell* **96**, 639–655.

Rodriguez-Navarro, S., Fischer, T., Luo, M.J., Antunez, O., Perez-Ortin, J.E., Reed, R., Hurt, E. (2004). Sus1, a functional componenet of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. *Cell* **116**, 75–86.

Sherman, F. (1991). Getting started with yeast. *Methods Enzymol.* **194**,3-21.

Sterner D., *et al.* (2002) SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. *Proc Natl Acad Sci U S A* **99**, 11622-7.

Tseng, S. S., Weaver, P. L., Liu,Y., Hitomi, M., Tartakoff, A. M., and Chang, T.-H. (1998) Dbp5p, a cytosolic RNA helicase, is required for poly(A)$^+$ RNA export. *EMBO J.* **17**, 2651–2662.

Weirich, C. S., *et al.* (2006). Activation of the DExD/H-box protein Dbp5 by the nuclear-pore protein Gle1 and its coactivator $InsP_6$ is required for mRNA export. *Nat. Cell Biol.* **8**, 668 – 676.

Strain	Genotype	Source
YTC279	MATα ade2 ade3 dbp5::HIS3 leu2 ura3 his3 trp1-Δ1 pCA5005 (=DBP5/LEU2).	This lab
YTC280	MATa ade2 ade3 dbp5::HIS3 leu2 ura3 his3 trp1-Δ1 pCA5005 (=DBP5/LUE2)	This lab
YTC1104	MATα ade2 ade3 dbp5::HIS3 leu2 ura3 his3 lys2-801 trp1-Δ1 pCA5061(=dbp5-1/TRP1) pCA5063 (=DBP5/ADE3/URA3)	This lab
YTC1202	MATa spt7::KAN ura3Δ leu2Δ his3Δ met15Δ LYS2	Open Biosystem
YTC1204	MATa thp1::KAN ura3Δ leu2Δ his3Δ met15Δ LYS2	Open Biosystem
YTC1205	MATa fen1::KAN ura3Δ leu2Δ his3Δ met15Δ LYS2	Open Biosystem
YTC1206	MATa rbk1::KAN ura3Δ leu2Δ his3Δ met15Δ LYS2	Open Biosystem
YTC1207	MATa pho1::KAN ura3Δ leu2Δ his3Δ met15Δ LYS2	Open Biosystem
YTC39	MATa mating type tester strain	S. Emr's lab
YTC40	MATα mating type tester strain	S. Emr's lab

Plasmid	Construction	Source
pCA5022	~2.6 Kb PCR product of *DBP5* was cloned into (*Bam*HI and *Sac*I).	This lab
pCA5024	Isolated Ts$^-$ *dbp5-1* allele in pRS315 backbone.	This lab
pRS315	Empty plasmid with LEU2 marker.	This lab
pTHP1001	~2.3 Kb THP1 PCR fragment was cloned into pRS315 (NotI and SacII).	This study
pRRP43001	~2.1 Kb RRP43 PCR fragment was clones into pRS315 (NotI and SacII).	This study
pSPT7001	~4.8 Kb SPT7 PCR fragment was cloned into pRS315 (NotI and SacII).	This study
pFEN1001	~1.9 Kb FEN1 PCR fragment was cloned pRS315 (NotI and SacII).	This study
pBUD5	~2.7 Kb BUD5 fragment was cloned into YCp50 (BamHI and XbaI).	Park's Lab

Table 1. Yeast strains and plasmids used

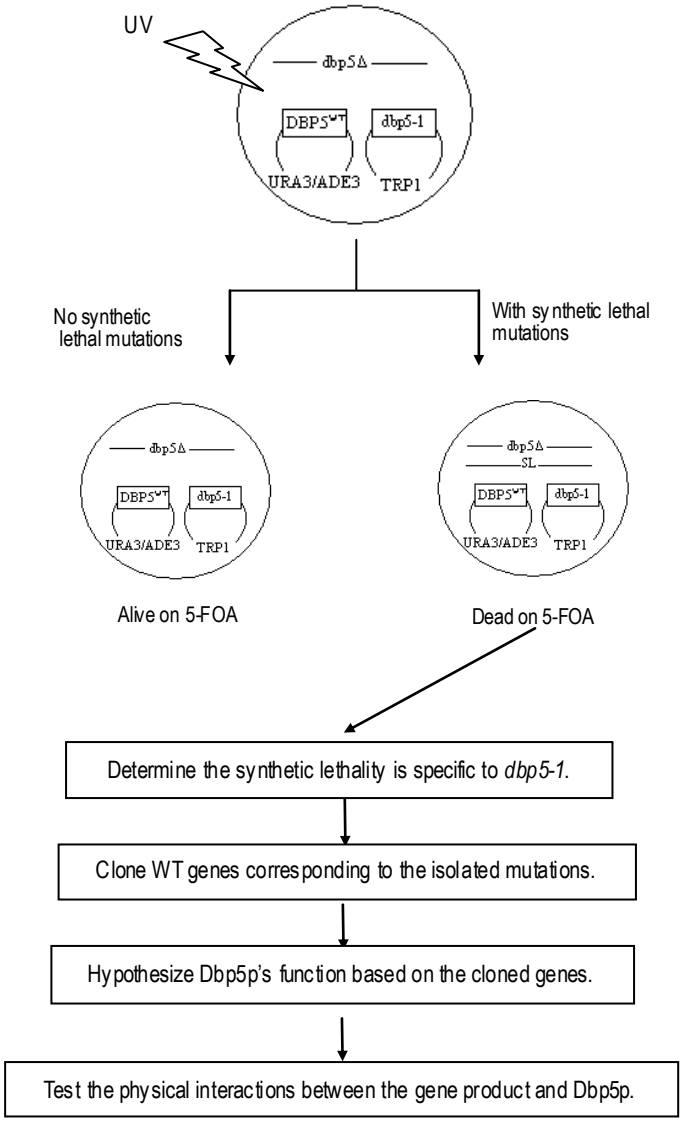


Figure 1. Schematic depicting the synthetic lethal screen and the following analyses.

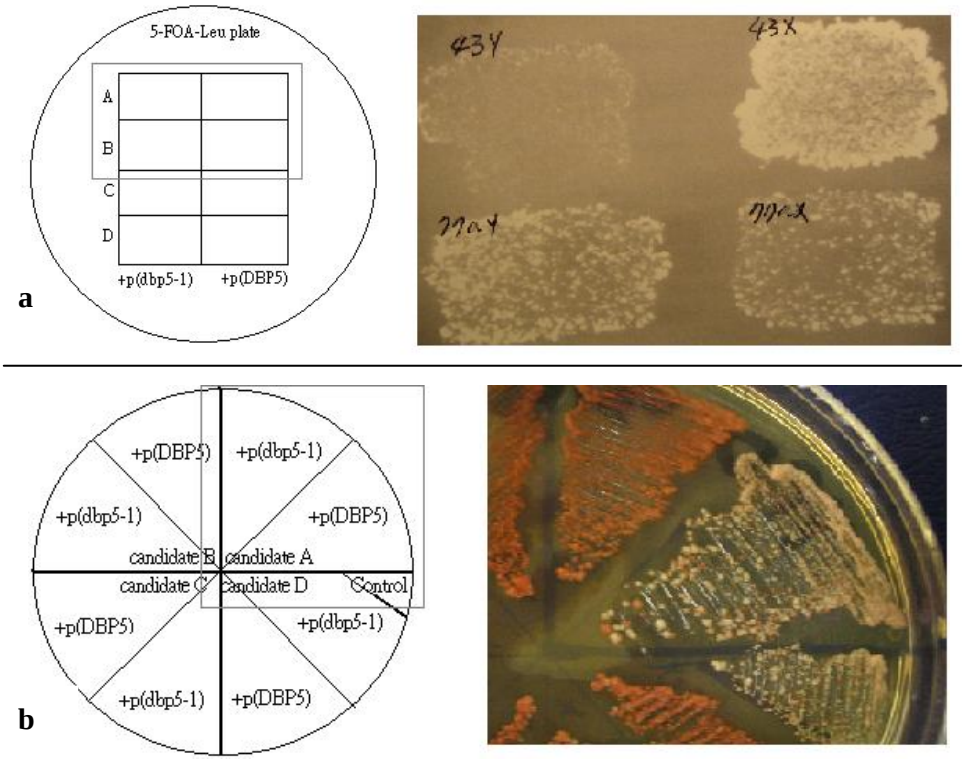


Figure 2. Phenotypes of isolated synthetic-lethal mutants upon transformations. (a) Candidate *A (*sl43*)* is a desired mutant that cannot grow on 5-FOA plate when acquiring *dbp5-1*, whereas candidate *B* is not a desired mutant. (b) On YPD plate, candidate A displayed a mixture of red and white colonies, when received an extra copy of DBP5.

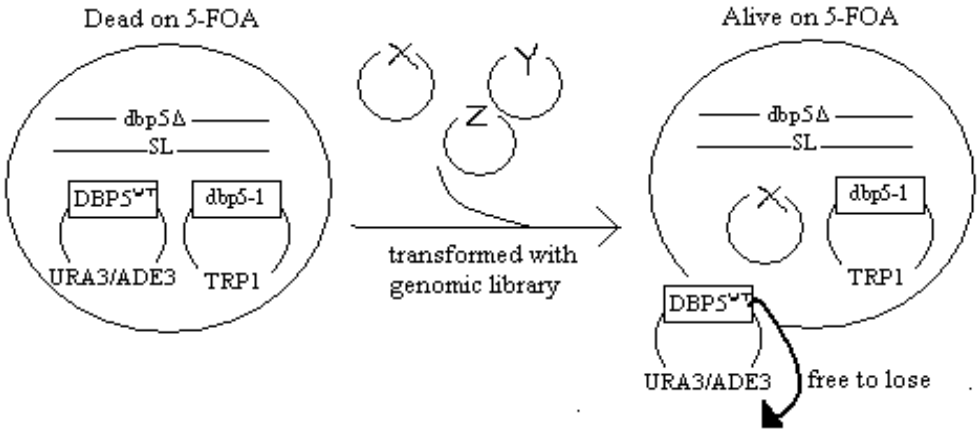


Figure 3. Illustration of genomic library transformation and screening. The isolated mutation strain is transformed by YCp50 library plasmids which contain different region insertions of yeast genomic DNA. When plasmid containing X was present in the cell, the *URA3* plasmid causing dead phenotype on 5-FOA plate can be freely lost. X represents the corresponding wild type gene region to the synthetic lethal mutation (SL mutation).

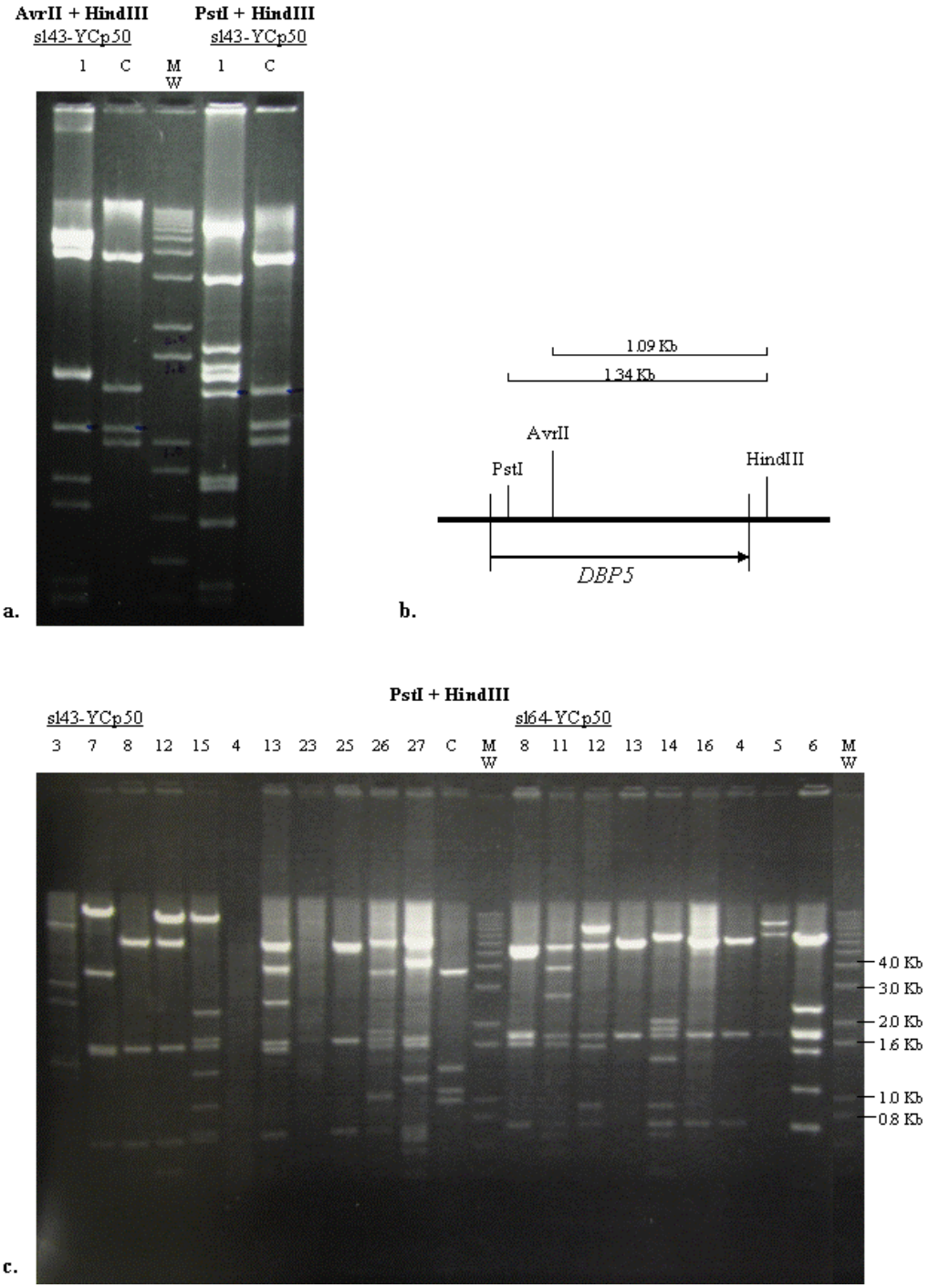


Figure 4. Results of restriction enzyme digestion of YCp50 clones. (a) *sl43-1* YCp50 and control were double digested under AvrII+HindIII or PstI+HindIII. The control plasmid contained a *DBP5* region. Common size bends of 1.09 Kb and 1.34 Kb between control and *sl43-1* YCp50 clone suggested that the obtained *sl43-1* YCp50 was actually a wild-type *DBP5* clone. The restriction sites were illustrated in (b). (c) More sl43-YCp50 and sl43-YCp50 restriction patterns by PstI+HindIII were shown here. Empty YCp50 plasmids gave bends at 0.77, 1.8, and 5.81 Kb. They would then be eliminated from the identification process. Only clones that were confirmed not a *DBP5* and patterns containing 0.77, 1.8, and other sizes bends depending on the DNA insertion after treated with PstI and HindIII, would go to retransformation verification.

SL Stains	Dom./ Rec.	Mutation Region	Possible Gene Identities
22	Dominant	--	--
27	Recessive	Chromosome II	*SPT7*
43	Recessive	Chromosome XV	*THP1*
47	Dominant	--	--
64	Recessive	Chromosome III	*FEN1, RRP43, BUD5*

Figure 5. Summary of isolated mutations and their possible identities of mutated genes.

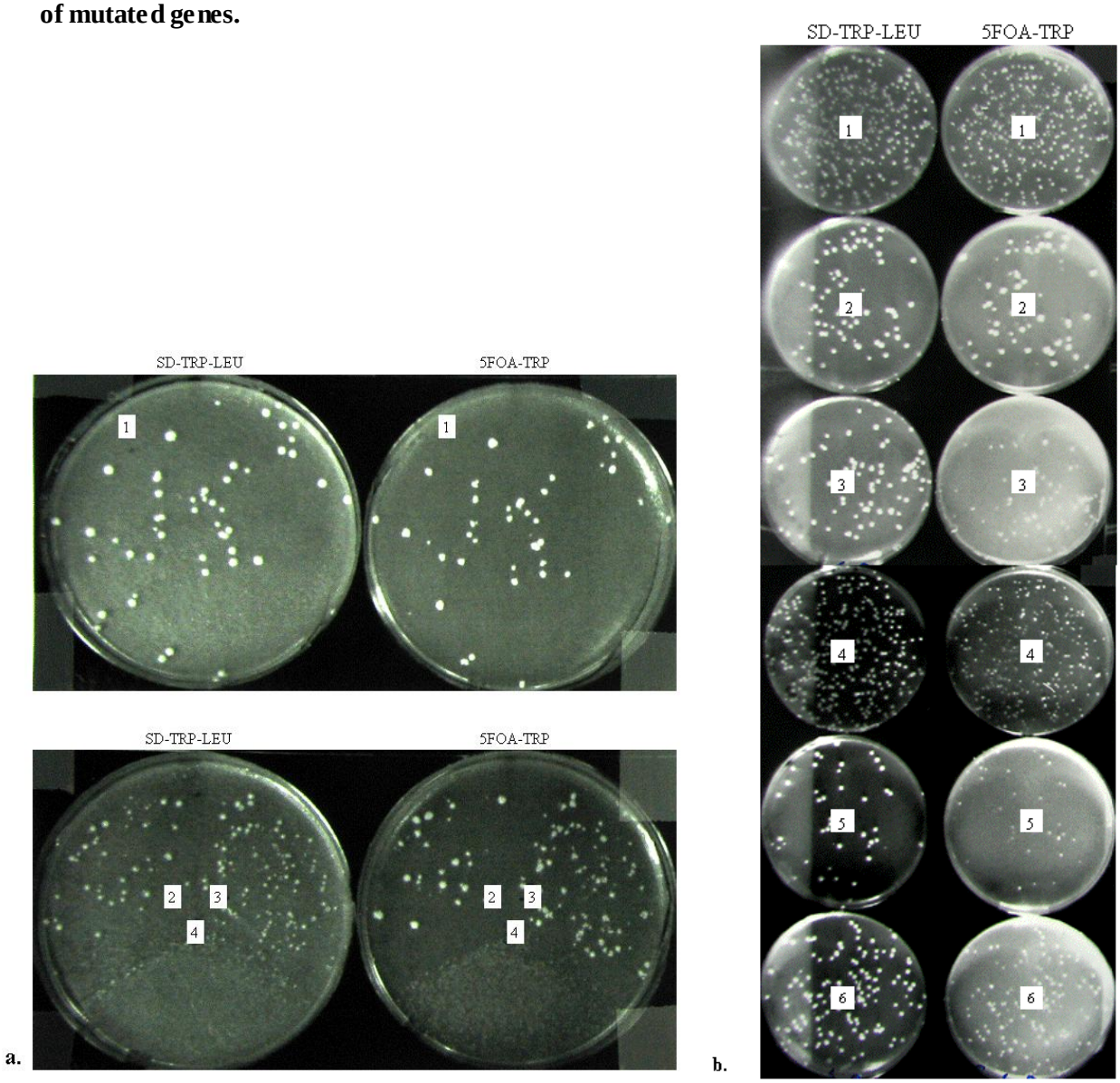
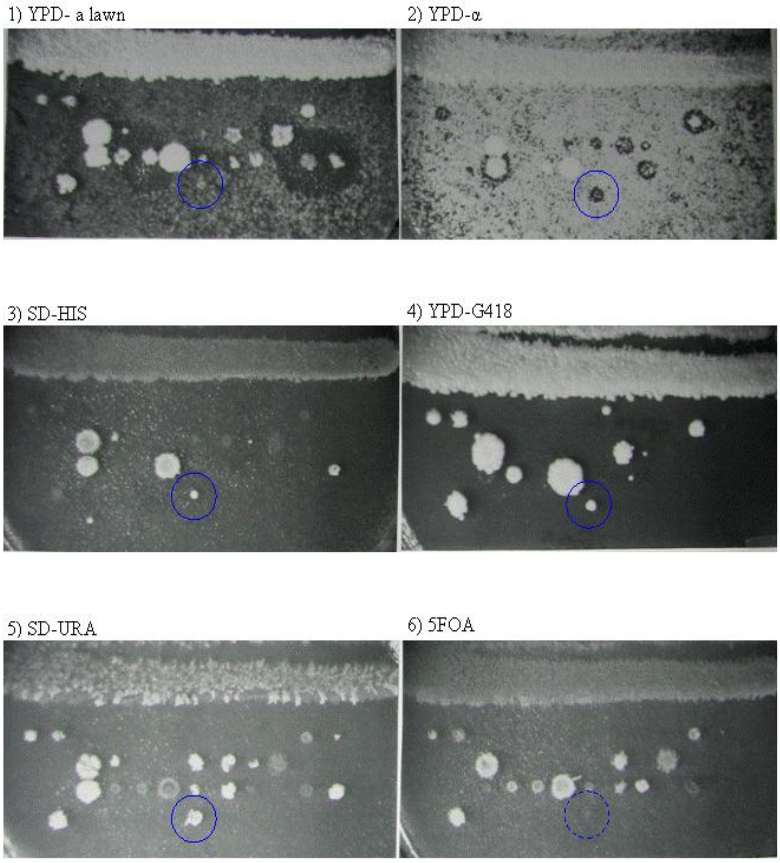
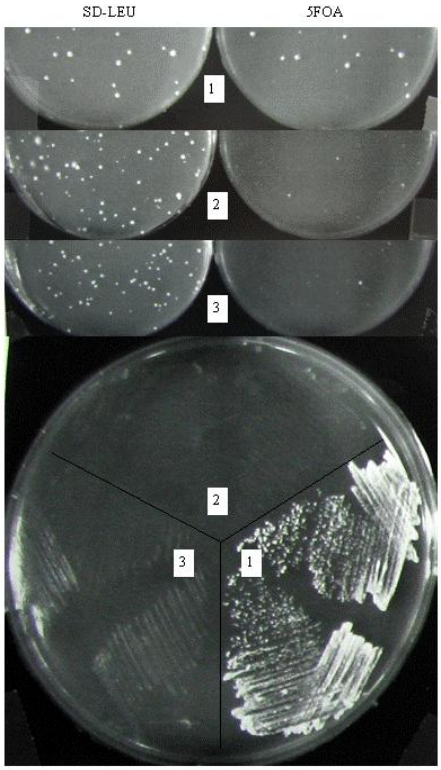


Figure 6. Retransformation with cloned gene and controls. (a) Retransformation of sl43 with 1: *THP1*, 2: *DBP5*, 3: sl43-15 YCp50, and 4: empty plasmid. Each of these plasmids held a *LEU2* selectable marker. 40 out of 42 (95%) *THP1* transformants could grow on 5-FOA while positive (2&3) and negative (4) controls were well-behaved. (b) Retransformation of sl64 with 1: *sl64-5* YCp50, 2: *DBP5*, 3: empty plasmid, 4: *BUD5*, 5: *RRP43*, and 6: *FEN1*. Each of these plasmids also held a *LEU2* selectable marker. The rescuing rate observed after three days on 5-FOA were 93%, 80%, 59%, 73%, 60%, and 60%, respectively.



a.



b.

Figure 7. Verification of the synthetic lethality of *thp1*Δ to *dbp5-1*. (a) Construction of chromosomal *dbp5*Δ and *thp1*Δ strain shown on replica-plates. Diploid formed from YTC279 and YTC1204 were dissected on YPD to build a spore containing both *dbp5*Δ and *thp1*Δ and keeping its copy of *DBP5* on a *URA3* plasmid. The criteria were HIS^+, $G418^+$, URA^+, and $5FOA^-$ phenotypes. The circled spore (*MATa*) was one of the double deletion strain candidates. (b) Transformation of the double deletion strain with 1: pCA5022 (*DBP5*), 2: pCA5024 (*dbp5-1*), 3: empty plasmid. The rescuing rates were 69%, 2%, and 1%, respectively. In this strain, the acquisition of a pCA5022 can rescue the cell on 5FOA, whereas pCA5024 cannot.